

I. AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions and listings. Claims 1-26 are currently pending in this application. Claim 1 has been amended.

Listing of Claims:

Claim 1 (currently amended) A method of manipulating the metabolism of a cell, comprising elevated expression of one or more enzymes involved in A-CoA metabolism, wherein said one or more enzymes are selected from the group consisting of alcohol acetyltransferase (AAT), alcohol acetyl transferase 2 (ATF2), pantothenate synthetase (PanC), aspartate 1-decarboxylase (PanD), pantothenate kinase (PanK), phosphopantetheine adenylyltransferase (CoAD), pyruvate dehydrogenase (PDH), pyruvate formate lyase (PFL), pyruvate oxidoreductase (POR), and combinations thereof, wherein said one or more enzymes are involved in one or more rate limiting steps of A-CoA synthesis.

Claim 2 (original) The method of claim 1, wherein the enzymes are selected from the group consisting of pyruvate dehydrogenase, pyruvate formate lyase, pyruvate oxidoreductase, pantothenate kinase, phosphopantetheine adenylyltransferase and combinations thereof.

Claim 3 (original) The method of claim 2, where the cell expresses one of the group consisting of i) overexpresses pantothenate kinase; ii) overexpresses pantothenate kinase and pyruvate dehydrogenase; iii) overexpresses pantothenate kinase where the panK gene is under the control of the lac promoter and additionally overexpresses the ATF2 gene under the control of the ptb promoter; and iii) overexpresses pantothenate kinase expression plasmid where the panK gene is under the control of the lac promoter and additionally overexpressing the ATF2 gene under the control of the ptb promoter, and pyruvate dehydrogenase.

Claim 4 (original) A method of increasing the A-CoA flux in a cell comprising elevated expression of one or more enzymes involved in A-CoA metabolism, wherein said one or more enzymes are involved in one or more rate limiting steps of A-CoA synthesis.

Claim 5 (original) The method of claim 4, wherein the enzymes are selected from the group consisting of pyruvate dehydrogenase, pyruvate formate lyase, pyruvate oxidoreductase, pantothenate kinase, phosphopantetheine adenylyltransferase and combinations thereof.

Claim 6 (original) A method of manipulating the metabolism of a cell, comprising deletion of one or more A-CoA utilizing pathways.

Claim 7 (original) The method of claim 6, wherein said one or more A-CoA utilizing pathways are selected from the group consisting of acetate formation pathway, citrate synthase formation pathway, fatty acid biosynthesis pathway, malonate formation pathway, and acetoacetate formation pathway.

Claim 8 (original) A method of increasing the A-CoA pools in a cell comprising deletion of one or more A-CoA utilizing pathways.

Claim 9 (original) The method of claim 8, wherein said one or more A-CoA utilizing pathways are selected from the group consisting of acetate formation pathway, citrate synthase formation pathway, fatty acid biosynthesis pathway, malonate formation pathway, and acetoacetate formation pathway.

Claim 10 (original) A method for the biosynthesis of one or more target compounds comprising increasing the intracellular levels of A-CoA and directing the increased A-CoA levels towards the biosynthesis of said one or more target compounds.

Claim 11 (original) The method of claim 10, wherein the intracellular levels of A-CoA are increased by elevated expression of one or more enzymes involved in A-CoA metabolism.

Claim 12 (original) The method of claim 10, wherein the intracellular levels of A-CoA are increased by deletion of one or more A-CoA utilizing pathways.

Claim 13 (original) The method of claim 10 wherein said one or more target compounds are selected from the group consisting of succinate, isoamyl alcohol, isoamyl acetate, esters, PHBs and polyketides.

Claim 14 (original) A method of producing isoamyl acetate in a cell comprising expressing at elevated levels one or more enzymes involved in A-CoA metabolism, wherein said cell displays increased flux through the A-CoA node.

Claim 15 (original) The method of claim 14 wherein said one or more enzymes are involved in one or more rate limiting steps of A-CoA synthesis.

Claim 16 (original) The method of claim 15, wherein the one or more enzymes are selected from the group consisting of pyruvate dehydrogenase, pyruvate formate lyase, pyruvate oxidoreductase, pantothenate kinase, phosphopantetheine adenylyltransferase and combinations thereof.

Claim 17 (original) A microorganism which expresses one or more enzymes involved in A-CoA metabolism at elevated levels, wherein said microorganism displays increased flux through the A-CoA node.

Claim 18 (original) The microorganism of claim 17, wherein said one or more enzymes are involved in one or more rate limiting steps of A-CoA synthesis.

Claim 19 (original) The microorganism of claim 18, wherein the one or more enzymes are selected from the group consisting of pyruvate dehydrogenase, pyruvate formate lyase, pyruvate oxidoreductase, pantothenate kinase, phosphopantetheine adenylyltransferase and combinations thereof.

Claim 20 (original) The microorganism of claim 17, wherein said microorganism is selected from the group consisting of ATCC _____, _____, _____, _____, _____, _____.

Claim 21 (original) A method of increasing CoA pools, comprising producing increased levels of pantothenate kinase (PanK) activity in a cell together with providing increased pantothenic acid levels, sufficient to increase the pool of CoA in the cell.

Claim 22 (original) The method of claim 21, wherein producing increased levels of PanK activity is achieved by transforming the cell with a vector that overexpresses the PanK gene and increased pantothenic acid is provided in a medium used to grow the cells.

Claim 23 (original) The method of claim 21, wherein producing increased levels of PanK activity is achieved by manipulating the host genome to overexpress the PanK gene and increased pantothenic acid is provided in the cell medium.

Claim 24 (original) A method of increasing synthesis of CoA containing compounds from a bacterial cell, comprising producing increased levels of pantothenate kinase (PanK) activity in a cell together with providing increased pantothenic acid levels, sufficient to increase the pool of CoA in the cell and drive the synthesis of CoA containing compounds.

Claim 25 (original) The method of claim 24, wherein producing increased levels of PanK activity is achieved by transforming the cell with a vector that overexpresses the PanK gene and increased pantothenic acid is provided in a medium used to grow the cells.

Claim 26 (original) The method of claim 24, wherein producing increased levels of PanK activity is achieved by manipulating the host genome to overexpresses the PanK gene and increased pantothenic acid is provided in the cell medium.